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FINAL REPORT

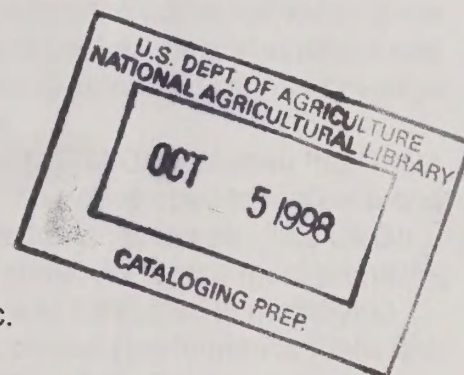
Cooperative Research and Development Agreement No. 58-3K95-6-456

*"Evaluation and Multiplication of Advanced Pinkeye-type Southernpea Breeding Lines
with Green Cotyledons"*

(June 1, 1996 through May 31, 1998)

submitted to

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Introduction and Justification

The development of southernpea [*Vigna unguiculata* (L.) Walp.] cultivars with a persistent green seed color has been the subject of much interest in recent years because the seeds of such cultivars can be harvested at the dry seed stage of maturity without loss of their fresh green color. The retention of the green color is important because the choice of harvest method is a compromise between cost and product quality. Mechanically harvested peas are usually harvested at a near-dry stage of maturity, and the harvested product contains a much lower percentage of green peas. A generation ago, peas were harvested at earlier maturity stages and the product quality was much higher.

In 1993, Fery et al. discovered a green cotyledon mutant in the cream-type cultivar Carolina Cream, and released a green cotyledon selection as the cultivar Bettergreen. Fery and Dukes (1994) reported that the green cotyledon trait exhibited by 'Bettergreen' is conditioned by a single recessive gene. They designated this gene *green cotyledon*, symbolized *gc*.

In the early 1990's, researchers at the U. S. Vegetable Laboratory, a USDA-ARS laboratory located at Charleston, S.C., initiated efforts to incorporate the green cotyledon gene into pinkeye-type southernpeas. The pinkeye is the predominant cultivar class of southernpea grown in the U.S. By the mid-1990's the USDA researchers had developed a series of pinkeye-type advanced breeding lines with green cotyledon phenotypes.

This Cooperative Research and Development Agreement (CRADA) between the USDA and Western Seed Multiplication, Inc. (WSM), Oglethrope, GA, was developed to evaluate and multiply advanced pinkeye-type breeding lines with green cotyledon phenotypes. The USDA was interested in applying for Plant Variety Protection (PVP) Certificates for the most promising lines, and WSM, as a potential licensee of such plant material, was interested in multiplying seed of the USDA's most advanced breeding lines, conducting on-farm performance trials, and arranging for evaluation of the suitability of harvested products therefrom for commercial processing. This report summarizes the results of the USDA research efforts under this two year (June 1, 1996 thru May 31, 1998) agreement.

USDA Responsibilities and Results

1. The USDA provided WSM seed stock of seven advanced generation (F_{10} and F_{11}) pinkeye-type southernpea breeding lines (candidate cultivars) homozygous for the green cotyledon phenotype (*gc/gc*). The USDA worked closely with WSM in planning all seed multiplication and evaluation activities outlined in the CRADA for execution by WSM. The USDA provided WSM assistance in conducting an inoculated field evaluation of all seven candidate cultivars for resistance to blackeye cowpea mosaic virus, and a USDA scientist visited the WSM research facility in Oglethrope, GA, while the evaluation work was in progress.
2. The USDA evaluated all seven of the candidate pinkeye-type cultivars for resistance to the southern root-knot nematode in its greenhouse research facility in Charleston, S.C. All of the lines were susceptible. [Note: Most of the leading pinkeye-type southernpea cultivars grown commercially in the U.S. are susceptible to southern root-knot nematodes.]

3. The USDA conducted extensive field evaluations of the pinkeye-type candidate cultivars during the spring and summer seasons of 1996 and 1997 at Charleston, S.C. Three 10-replicate tests and two 5-replicate tests were conducted in 1996 and two 10-replicate tests were conducted in 1997. Additionally, extensive greenhouse and field plantings were grown to develop adequate quantities of breeder's seed of several lines. Two of the candidate cultivars (US-858, Charleston Greenpack; US-861, Petite-N-Green) proved to be quite promising in these tests, and an extensive data base was collected to support the preparation of both USDA cultivar release documentation and Plant Variety Protection Certificates (Tables 1 - 5).
4. Based upon results of work conducted by both the USDA and WSM, the USDA prepared cultivar release notices and applications for Plant Variety Protection Certificates for two pinkeye-type southernpea cultivars with green cotyledon phenotypes. Experimental line US-858 was approved for release on May 2, 1997 as 'Charleston Greenpack' (Attachment #1); the application for plant variety protection is pending. A formal request was made on June 8, 1998 to release Experimental line US-861 as 'Petite-N-Green'. The official release notice (Attachment #2) and application for plant variety protection for 'Petite-N-Green' are pending.

Summary

This Cooperative Research and Development Agreement (CRADA) between the USDA and Western Seed Multiplication, Inc. (WSM), Oglethorpe, GA, was developed to evaluate and multiply advanced pinkeye-type southernpea breeding lines with green cotyledon phenotypes. The USDA had initiated a breeding program in the early 1990's to develop such materials and had seven "candidate" cultivars ready for testing. The USDA was interested in applying for Plant Variety Protection Certificates for the most promising of the lines, and WSM, as a potential licensee of such material, was interested in multiplying seed of the most promising lines, conducting on-farm performance trials, and arranging for evaluation of the suitability of harvested products therefrom for commercial processing. This report documents the efforts made by the USDA under this agreement. These efforts have resulted in the development of an extensive data base on two promising pinkeye-type southernpea breeding lines with green cotyledon phenotypes. One of these breeding lines (US-858) was released in 1997 as the cultivar Charleston Greenpack, and release of the second breeding line (US-861) as the cultivar Petite-N-Green is pending.

Literature Cited

- Fery, R. L., and P. D. Dukes. 1994. Genetic analysis of the green cotyledon trait in southernpea [*Vigna unguiculata* (L.) Walp.]. J. Amer. Soc. Hort. Sci. 119:1054-1056.
- Fery, R. L., P. D. Dukes, and F. P. Maguire. 1993. 'Bettergreen' southernpea. HortScience 28:856.

Table 1. Plant height, plant width, peduncle length, pod length, percent machine shellout, and number of peas per pod for 'Charleston Greenpack', 'Coronet', 'Kiawah', and 'Pinkeye Purple Hull-BVR' southernpeas grown in a late spring planting, Charleston, S.C., 1996.

Cultivar	Plant height (cm)	Plant width (cm)	Peduncle length (cm)	Pod length (cm)	Machine shellout ^z (%)	Peas/pod (no.)
Charleston Greenpack	43.6 b ^y	68.8 b	31.3 b	17.3 b	78.9 a	14.3 a
Coronet	47.1 a	73.8 a	33.1 a	16.4 c	77.8 b	13.2 b
Kiawah	45.8 a	71.5 ab	34.4 a	17.5 b	77.6 b	13.6 b
Pinkeye Purple Hull-BVR	46.0 a	70.6 b	34.6 a	18.2 a	76.5 c	13.6 b

^zPercentage of dry seed obtained from machine-shelled sample of dry pods [(mass of dry seed/mass of dry pods) x 100].

^yMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Table 2. Fresh and dry seed characteristics of 'Charleston Greenpack', 'Coronet', 'Kiawah', and 'Pinkeye Purple Hull-BVR' southernpeas, Charleston, S.C., 1996.

Cultivar	Fresh			Dry		
	Length ^z (mm)	Width ^z (mm)	Mass/ 100 peas ^y (g)	Length ^y (mm)	Width ^y (mm)	Mass/ 100 peas ^y (g)
Charleston Greenpack	10.6 c ^x	6.0 b	29.8 a	7.0 b	4.2 b	12.8 b
Coronet	10.8 bc	6.4 a	31.3 a	7.2 a	4.4 a	13.2 a
Kiawah	10.9 b	6.1 b	30.3 a	7.0 b	4.4 a	13.4 a
Pinkeye Purple Hull-BVR	11.2 a	6.0 b	31.0 a	7.1 ab	4.3 ab	13.5 a

^zPea samples harvested from replicated field test planted 12 June.

^yPea samples harvested from replicated field test planted 23 May.

^xMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Table 3. Dry seed yields of 'Charleston Greenpack', 'Coronet', 'Kiawah', and 'Pinkeye Purple Hull-BVR' southernpeas grown in spring, late spring, and summer plantings, Charleston, S.C., 1996^z.

Cultivar	Dry seed yield (kg.ha ⁻¹)		
	Spring	Late spring	Early summer
Charleston Greenpack	1307 a ^y	1096 a	488 a
Coronet	1516 a	1068 ab	615 a
Kiawah	1429 a	953 c	504 a
Pinkeye Purple Hull-BVR	1329 a	986 bc	545 a

^zSpring, late spring, and early summer tests planted on 23 May, 12 June, and 2 July, respectively. The experimental design of each test was a randomized complete block with 10 replications. Each plot was space-planted, 18 hills per plot, three seeds per hill, 30 cm between hills, and 102 cm between rows. Single harvest of dry pods.

^yMean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

Table 4. Plant height, plant width, peduncle length, pod length, and number of peas per pod for 'Petite-N-Green', 'Charleston Greenpack', 'Coronet', and 'Pinkeye Purple Hull-BVR' southernpeas, Charleston, S.C., 1997.

Cultivar	Plant height (cm) ^z	Plant width (cm) ^z	Peduncle length (cm) ^z	Pod length (cm) ^y	Peas/ pod (no.) ^y
Petite-N-Green	49.8 a*	79.3 a	35.5 a	14.4 c	13.7 a
Charleston Greenpack	47.9 a	67.2 c	34.1 a	15.3 b	11.6 b
Coronet	51.3 a	76.3 a	36.6 a	15.1 b	11.5 b
Pinkeye Purple Hull-BVR	47.8 a	71.0 b	36.0 a	16.0 a	11.1 b

^zSpring test. Experimental procedures described in Table 5 footnote.

^ySummer test. Experimental procedures described in Table 5 footnote.

*Mean separation within columns by Duncan's multiple range test.

Table 5. Number of days to harvest, machine shellout, mass per 100 dry peas, and dry pea yield for 'Petite-N-Green', 'Charleston Greenpack', 'Coronet', and 'Pinkeye Purple Hull-BVR' southernpeas grown in spring and summer plantings, Charleston, S.C., 1996 and 1997².

Cultivar	Days to harvest (no.)	Machine shellout (%) ^y	Mass/100 dry peas (g)	Dry pea yield (kg.ha ⁻¹)
<i>Spring 1996</i>				
Petite-N-Green	71.0 a ^x	80.7 a	10.3 b	1,121 a
Charleston Greenpack	67.0 a	78.2 b	12.9 a	978 a
Coronet	66.4 a	79.8 a	13.1 a	1,175 a
Pinkeye Purple Hull-BVR	66.0 a	76.9 c	13.4 a	1,259 a
<i>Summer 1996</i>				
Petite-N-Green	69.6 a	75.7 b	9.7 c	699 a
Charleston Greenpack	62.4 b	79.1 a	12.2 a	586 a
Coronet	67.8 a	71.6 c	11.1 b	548 a
Pinkeye Purple Hull-BVR	68.0 a	72.9 bc	11.6 ab	586 a

Spring 1997

Petite-N-Green	70.0 a	79.6 a	10.1 c	761 b
Charleston Greenpack	65.0 c	78.1 b	12.6 b	967 a
Coronet	66.2 b	78.9 ab	13.4 a	971 a
Pinkeye Purple Hull-BVR	66.9 b	74.1 c	13.3 a	732 b

Summer 1997

Petite-N-Green	75.8 a	80.0 a	13.1 b	719 ab
Charleston Greenpack	68.6 b	77.6 a	14.8 a	592 b
Coronet	67.6 b	78.2 a	14.7 a	869 a
Pinkeye Purple Hull-BVR	66.6 b	74.4 b	14.9 a	596 b

²Spring and summer 1996 tests planted on 23 May and 2 July, respectively.

Spring and summer 1997 tests planted on 2 June and 25 June, respectively. The experimental design of each test was a randomized complete block with 5 (1996) or 10 replications (1997). Each plot was space-planted, 18 hills per plot, three seeds per hill, 30 cm between hills, and 102 cm between rows. Single harvest of dry pods.

³Percentage of dry seed obtained from machine-shelled sample of dry pods
 $[(\text{mass of dry seed} / \text{mass of dry pods}) \times 100]$.

⁴Mean separation within columns by Duncan's multiple range test at $P \leq 0.05$.

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Washington, DC 20250

NOTICE OF RELEASE OF 'CHARLESTON GREENPACK'
PINKEYE-TYPE SOUTHERNPEA

The Agricultural Research Service, U. S. Department of Agriculture, announces the release of CHARLESTON GREENPACK southernpea (*Vigna unguiculata*). CHARLESTON GREENPACK is the first pinkeye-type southernpea cultivar to be developed that exhibits the green cotyledon trait. The new cultivar can be harvested at the near-dry stage of maturity without loss of the pea's fresh green color. CHARLESTON GREENPACK was developed at the U.S. Vegetable Laboratory, Charleston, SC, by Dr. Richard L. Fery, Research Geneticist.

CHARLESTON GREENPACK was developed over a 7-year period using a backcross-pedigree breeding procedure that included two hybridizations and repeated single-plant selections. The initial cross involved 'Bettergreen', a cream-type cultivar with green-colored cotyledons, and 'Kiawah', a pinkeye-type cultivar with cream-colored cotyledons. The second cross was a backcross of the F_1 of the initial cross to the 'Kiawah' parent. Following the last hybridization, intense selection pressure was applied in the F_2 through F_8 generations for the green cotyledon phenotype and superior horticultural characteristics. CHARLESTON GREENPACK originated as a bulk of an F_8 population grown in 1994.

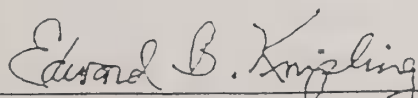
CHARLESTON GREENPACK is similar in appearance to 'Coronet' and 'Pinkeye Purple Hull-BVR', but usually matures two to three days earlier. The plant habit is low bushy and somewhat more compact than the plant habits of either 'Coronet' or 'Pinkeye Purple Hull-BVR'. There is extensive pigmentation (purple) on the stems, branches, and peduncles; the petioles contain moderate pigmentation at the base and tip. Flower color is predominately white; the upper, inside margin of the standard is lightly pigmented (violet), the base of the standard is yellow, and the wing is pigmented (violet) on the back surface. Pod set is concentrated, and the pods are borne above the foliage in a scattered fashion. Dry pods are attached to the peduncles in a pendant manner, and each peduncle typically produces two pods. A typical pod is moderately curved, 17 centimeters long, and contains 14 peas. Pod color is green (Munsell rating: 5 GY 4/6) when immature, dark purple (Munsell rating: 10 P 2/1) when ready for mature-green harvest, and dark purple (Munsell rating: 10 P 2/1) when dry. The upper sutures and tips of immature pods are pigmented. Fresh peas are kidney shaped and have a pink eye (Munsell rating: 2.5 R 5/6), quite similar to fresh 'Coronet' and 'Pinkeye Purple Hull-BVR' peas. The dry peas have a smooth seed coat, and are slightly smaller than those of 'Coronet' and 'Pinkeye Purple Hull-BVR' (12.8 vs. 13.2 and 13.5 grams/100 peas, respectively). Results of spring, late spring, and early summer 10-replicate field tests conducted at Charleston, SC, indicate that CHARLESTON GREENPACK yields are comparable to 'Coronet' yields, and comparable to or better than 'Pinkeye Purple Hull-BVR' yields.

CHARLESTON GREENPACK is homozygous for the *gc* gene that conditions the green cotyledon trait. Seed coats and cotyledons of field-grown CHARLESTON GREENPACK peas harvested at dry-stage maturity are light olive in color (Munsell rating: 7.5 Y 7/4). In comparison, seed coats and cotyledons of 'Coronet' and 'Pinkeye Purple Hull-BVR' peas harvested at similar maturity have a cream color (Munsell rating: 2.5 Y 8/4). Dry CHARLESTON GREENPACK peas imbibed to restore fresh-harvest seed size and blanched in boiling water for three minutes exhibit a "fresher" color than dry seeds. Imbibed and blanched CHARLESTON GREENPACK peas have a near-fresh green color (Munsell rating: 10 Y 7/4); similarly treated 'Coronet' and 'Pinkeye Purple Hull-BVR' peas have a cream color (Munsell rating: 5 Y 8/2). The foliage of field-grown CHARLESTON GREENPACK plants tends to have a greener color than the foliage of 'Coronet' and 'Pinkeye Purple Hull-BVR' plants. Additionally, the inside surface of "mature-green" CHARLESTON GREENPACK pod hulls has a greener color than inside surface of the pod hulls of the other pinkeye cultivars.

CHARLESTON GREENPACK was grown "on-farm" in Georgia and Florida during the 1996 crop season by Western Seed Multiplication, Inc., Oglethorpe, GA. The purpose of these plantings was to multiply seed, to evaluate field performance, to arrange for evaluation of the suitability of harvested products for commercial processing, and to test for field resistance to blackeye cowpea mosaic virus (BICMV). CHARLESTON GREENPACK performed well in all instances. The harvested product produced a frozen pack with excellent characteristics, and the results of inoculated field plantings indicate that the new cultivar has excellent field resistance to BICMV.

CHARLESTON GREENPACK is recommended for use as a home-garden and fresh-market cultivar for spring, mid-season, and fall plantings throughout the southern United States. It should perform well in all areas where 'Coronet' or 'Pinkeye Purple Hull-BVR' has been grown successfully. CHARLESTON GREENPACK is recommended particularly as a processing cultivar for the frozen-food industry.

Protection for CHARLESTON GREENPACK will be sought under the Plant Variety Protection Act. CHARLESTON GREENPACK was developed under a Cooperative Research and Development Agreement with Western Seed Multiplication, Inc. (WSM), Box 173AA, Oglethorpe, GA 31068, and WSM has been granted the right of first refusal to an exclusive license to market the new cultivar. Breeder seed will be maintained by USDA-ARS, U.S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC 29414-5334. Genetic material of this release will be deposited in the National Plant Germplasm System where it will be available for research purposes, including development and commercialization of new cultivars.



Administrator, Agricultural Research Service
United States Department of Agriculture

MAY 6 2 1997

Date

DRAFT

**UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
WASHINGTON, D.C. 20250**

**NOTICE OF RELEASE OF 'PETITE-N-GREEN', A SMALL SEEDED, FULL SEASON,
GREEN COTYLEDON, PINKEYE-TYPE SOUTHERNPEA**

The Agricultural Research Service, U. S. Department of Agriculture, announces the release of PETITE-N-GREEN southernpea (*Vigna unguiculata*). PETITE-N-GREEN is a small-seeded, full season, large vined, pinkeye-type southernpea that exhibits the green cotyledon trait. The new cultivar produces excellent yields of small, delicate peas that can be harvested at the dry stage of maturity without loss of the pea's fresh green color. The unique combination of plant habit, maturity, and seed traits should make PETITE-N-GREEN more appealing to home gardeners than the recently released pinkeye-type, green cotyledon cultivar Charleston Greenpack that was developed for the frozen food industry. PETITE-N-GREEN was developed at the U. S. Vegetable Laboratory, Charleston, S.C., by Dr. Richard L. Fery, Research Geneticist.

PETITE-N-GREEN was developed over an 8-year period using a pedigree breeding procedure that included a single cross and repeated single-plant selections. The cross involved 'Bettergreen', a cream-type pea with green-colored cotyledons, and 'Coronet', a pinkeye-type cultivar with cream-colored cotyledons. Intense selection pressure was applied in the F₂ through the F₉ generations for the green cotyledon phenotype and superior horticultural characteristics. PETITE-N-GREEN originated as a bulk of an F₉ population grown in 1994.

PETITE-N-GREEN has a low bushy plant habit similar to that of 'Coronet'. It has a more procumbent vine than 'Charleston Greenpack'. PETITE-N-GREEN produces dry pods in 70 to 76 days, four to seven days later than 'Charleston Greenpack' and two to nine days later than 'Coronet' and 'Pinkeye Purple Hull-BVR'. There is extensive pigmentation (purple) on the peduncles; the stems, branches, and petioles contain slight to moderate pigmentation at the bases and tips. Flower color is predominately white; the upper, inside margin of the standard is lightly pigmented (violet), the base of the standard is yellow, and the wing is pigmented (violet) on the back surface. Pod set is concentrated, and the pods are borne at foliage level in a scattered fashion. Dry pods are attached to the peduncles in a pendant manner, and each peduncle typically produces two pods.

A typical PETITE-N-GREEN pod is moderately curved, 14 centimeters long, and contains 14 peas. Pod color is green when immature, dark purple when ready for mature-green harvest, and dark purple when dry. The upper sutures and tips of immature pods are pigmented. Fresh peas are ovate to kidney shaped and have a pink eye that is quite similar to those of fresh 'Charleston Greenpack', 'Coronet' and 'Pinkeye Purple Hull-BVR' peas. Results of replicated spring and summer field tests conducted at Charleston, S.C., during 1996 and 1997 indicate that PETITE-N-GREEN yields are usually comparable to 'Charleston Greenpack' and 'Coronet' yields, and comparable to or better than 'Pinkeye Purple Hull-BVR' yields. The dry peas are small (9.7 to 13.1 grams per 100 peas) and have a smooth seed coat. PETITE-N-GREEN peas are 11.5% to 20.5% smaller than 'Charleston Greenpack' peas, 10.9% to 24.6% smaller than 'Coronet' peas, and 12.1% to 24.1% smaller than 'Pinkeye Purple Hull-BVR' peas.

PETITE-N-GREEN is homozygous for the *gc* gene that conditions the green cotyledon trait. Seed coats and cotyledons of field-grown PETITE-N-GREEN peas harvested at the dry-stage of maturity are light olive in color (Munsell rating: 7.5 Y 7/4). Seed coats and cotyledons of 'Coronet' and 'Pinkeye Purple Hull-BVR' peas harvested at a similar stage of maturity have a cream color (Munsell rating: 2.5 Y 8/4). Dry PETITE-N-GREEN peas imbibed to restore fresh-harvest seed size and blanched in boiling water for three minutes exhibit a near-fresh green color (Munsell rating: 10 Y 7/4); similarly treated 'Coronet' and 'Pinkeye Purple Hull-BVR' peas have a cream color (Munsell rating: 5 Y 8/2).

PETITE-N-GREEN is recommended particularly for use as a home-garden cultivar in the southeastern United States. Not only can the peas be harvested fresh for immediate consumption or storage in home freezers, but they can also be harvested when fully dry and stored as an attractive dry pack. The dry peas can be removed from storage and imbibed to restore a near-fresh green color. PETITE-N-GREEN should perform well in areas where the popular home garden cultivar Pinkeye Purple Hull has been grown successfully.

Protection for PETITE-N-GREEN will be sought under the Plant Variety Protection Act. PETITE-N-GREEN was developed under a Cooperative Research and Development Agreement with Western Seed Multiplication, Inc. (WSM), Box 173AA, Oglethorpe, GA 31068, and WSM has been granted the right of first refusal to an exclusive license to market the new cultivar. Breeder's seed will be maintained by USDA-ARS, U. S. Vegetable Laboratory, 2875 Savannah Highway, Charleston, SC 29414-5334. Genetic material of this release will be deposited in the National Plant Germplasm System where it will be available for research purposes, including development and commercialization of new cultivars.

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